Dadapter

Document Number 1

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DOCUMENT-IDENTIFIER: US 5811269 A
TITLE: Detection of mycobacteria by multiplex nucleic acid amplification

DEPR:
The target binding sequences of the amplification primers and the adapter primers confer genus or species hybridization specificity and therefore provide the species- and genus -specificity to the assay. The target binding sequences are essential to obtain the species -specific/genus-specific multiplex amplification of the invention. By way of example, the IS6110 and 16S rDNA amplification primers listed above contain a recognition site for the restriction endonuclease BsoBI which is nicked during the SDA reaction. It will be apparent to one skilled in the art that other nickable restriction endonuclease recognition sites may be substituted for the BsoBI recognition site, including but not limited to those recognition sites disclosed in EP 0 684 315. Preferably, the recognition site is for a thermophilic restriction endonuclease so that the amplification reaction may be performed under optimum conditions. Similarly, the tail sequence of the amplification primer (5' to the restriction endonuclease recognition site) is generally not critical, although it is important to avoid including the restriction site used for SDA and to avoid sequences which will hybridize either to their own target binding sequence or to the other primers. The amplification primers of the invention therefore consist of the 3' target binding sequences indicated in Table I, a nickable restriction endonuclease recognition site 5' to the target binding sequence and a tail sequence about 10-25 nucleotides in length 5' to the restriction endonuclease recognition site. The length of the tail depends on the T.sub.m of the selected sequence, and can be easily adjusted by one skilled in the art to obtain sufficient hybridization. The amplification products of the IS6110, 16S rDNA and the internal control target sequences may be detected by hybridization to an oligonucleotide probe tagged with a detectable label, each target specifically hybridizing to a separately detectable probe. If the target-specific and control-specific probes are hybridized simultaneously to the amplification products, the labels should be separately identifiable to facilitate distinguishing the respective targets. Otherwise, separate aliquots of the amplification reaction may be hybridized to target -specific probes tagged with the same label. The detectable label may be conjugated to the probe after it is synthesized or it may be incorporated into the probe during synthesis, for example in the form of a label-derivatized nucleotide. Such labels are known in the art and include directly and indirectly detectable labels. Directly detectable labels produce a signal without further chemical reaction and include such labels as fluorochromes, radioisotopes and dyes. Indirectly detectable labels require further chemical reaction or addition of reagents to produce the detectable signal. These include, for example, enzymes such as horseradish peroxidase and

alkaline phosphatase, ligands such as biotin which are detected by binding to label-conjugated avidin, and chemiluminescent molecules. The probes may be hybridized to their respective amplification products in solution, on gels, or on solid supports. Following hybridization, the signals from the associated labels are developed, detected and optionally quantitated using methods appropriate for the selected label and hybridization protocol. The amount of signal detected for each amplification product indicates the relative amount of each amplification product present.

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l2 same (tetranucleotide$ or
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USPT	l3 same (tetranucleotide$ or pentanucleotide$ or hexanucleotide$ or septanucleotide$ or octanucleotide$)	0	L15
USPT	l7 same denatur$	0	L14
USPT	l3 same label$	88	L13
USPT	l3 same genotyp$	2	L12
USPT	l3 same reference	20	L11
USPT	l2 same reference	753	L10
USPT	l8 same (zinc near0 finger)	5	L9
USPT	l2 same protein	1319	L8
USPT	l2 same HPLC	13	L7
USPT	l2 same RAPD	6	L6
USPT	l3 same RAPD	0	L5
USPT	l3 same HPLC	0	L4
USPT	l2 same (solid near0 support)	190	L3
USPT	l1 same amplif$	5624	L2
USPT	sequenc$ same hybrid$	17727	L1